Constitutive Gs activation using a single-construct tetracycline-inducible expression system in embryonic stem cells and mice.

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ABSTRACT: INTRODUCTION: The controlled expression of many genes, including G-protein coupled receptors (GPCRs), is important for delineating gene functions in complex model systems. Binary systems for inducible regulation of transgene expression are widely used in mice. One system is the tTA/TRE expression system, composed of a tetracycline-dependent DNA binding factor and a separate tetracycline operon. However, the requirement for two separate transgenes (one for each tTA or TRE component) makes this system less amenable to models requiring directed cell targeting, increases the risk of multiple transgene integration sites, and requires extensive screening for appropriately-functioning clones. METHODS: We developed a single, polycistronic tetracycline-inducible expression platform to control the expression of multiple cistrons in mammalian cells. This platform has three basic constructs: regulator, responder, and destination vectors. The modular platform is compatible with both the TetOff (tTA) and TetOn (rtTA) systems. The modular Gateway-recombineering-compatible components facilitate rapidly generating vectors to genetically modify mammalian cells. We apply this system to use the elongation factor 1alpha (EF1alpha) promoter to drive doxycycline-regulated expression of both the fluorescent marker mCherry and an engineered Gs-coupled GPCR "Rs1" separated by a 2A ribosomal skip site. RESULTS: We show that our combined expression construct drives expression of both the mCherry and Rs1 transgenes in a doxycycline-dependent manner. We successfully target the expression construct into the Rosa26 locus of mouse embryonic stem (ES) cells. Rs1 expression in mouse ES cells increases cAMP accumulation via both basal and ligand-induced Gs mechanisms and is associated with increased embryoid body size. Heterozygous mice carrying the Rs1 expression construct showed normal growth and weight, and developed small increases in bone formation that could be observed in the calvaria. CONCLUSIONS: Our results demonstrate the feasibility of a single-vector strategy that combines both the tTA and TRE tetracycline-regulated components for use in cells and mouse models. Although the EF1alpha promoter is useful for driving expression in pluripotent cells, a single copy of the EF1alpha promoter did not drive high levels of mCherry and Rs1 expression in the differentiated tissues of adult mice. These findings indicate that promoter selection is an important factor when developing transgene expression models.